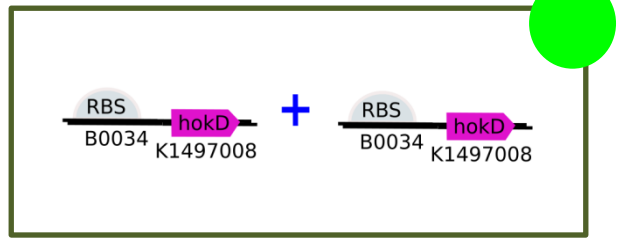


Assembly:

2X_RBS_hokD



1st Day:

EXSP Digestion (see **Enzymatic Digestion Protocol**)

	Part	Size	ηg/μl
1	RBS_hokD	168 bp	121.3
2	RBS_hokD	168 bp	121.3

	Volume to 1,0 μg (μl)	Buffer 10x (μl)	BSA	Enzyme 1	Volume (μl)	Enzyme 2	Volume (μl)	H ₂ O to 20μl (μl)
1	8.2	2 (M)	-	X	1	P	1	7.8
2	8.2	2 (M)	-	S	1	P	1	7.8

Final Plasmid	Resistance
pSB1A2	ampicillin

Gel purification

- See PureLink® Quick Gel Extraction Kit Invitrogen™ manual
- Quantify digestion products

Parts	ηg/μl
RBS_hokD	13.5
RBS_hokD	13.5

Obs: 260/280 in a quality parameter that tells you if your sample is contaminated with proteins. The greater it is compared to 1 the less contaminants you have.

Ligation (see **Ligation Protocol**)

Part containing the plasmid	RBS_hokD	3.7
Insert	RBS_hokD	1.0
10x T4 DNA Buffer	2	
T4 DNA ligase 1u	0.4	
H2O to 20μl	12.9	

Obs: To determinate the amount of DNA necessary we used the following equation

$$\text{Insert ng} = \text{plasmid ng} \times \text{insert bp} / \text{plasmid bp} \times \text{insert: plasmid ratio}$$

- Incubate overnight at 37°C.
- Prepare and sterilize in the autoclave tubes with 6 ml of liquid LB medium.
- Prepare glycerol 40%

2nd Day:

Transformation (see **Transformation Protocol in Escherichia coli DH5-α**)

- Organism: E. coli DH5-α
- Selection: Ampicillin

4th Day:

Confirmation with NotI